PROCESSING OF DNA HIGH-ANGLE FIBRE DIFFRACTION PATTERNS USING THE CCP13 SUITE

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The use of the CCP13 suite of programmes in the processing of high-angle x-ray diffraction data recorded from DNA fibres is described. Two examples have been chosen to demonstrate the application of these routines in the measurement of highly crystalline diffraction patterns. The first of these describes the processing of data recorded from the A conformation of a novel deaza derivative of the poly[d(A-T)].poly[d(A-T)] DNA double helix in which the N_7 position of adenine residues has been changed to a C-H group. The second example concerns the processing of fibre diffraction data which has recently been obtained from a thallium derivative of the D form of DNA.

Introduction High-angle fibre diffraction is a powerful method in the analysis of DNA structure and in the investigation of conformational changes between DNA structures. Fibre diffraction studies of long polymeric DNA and also single crystal studies of oligonucleotide duplexes have provided clear evidence that water and cations in these systems occupy well defined locations which are crucial both to the stabilisation of each of the five principal forms of double-stranded DNA and to the mediation of conformational transitions which occur between these forms. This is well illustrated by time-resolved high-angle fibre diffraction studies of structural transitions in DNA which have been undertaken at the Daresbury SRS[1,2,3]. These observations have led to the use of x-ray isomorphous replacment methods in the study of the location of cations around DNA and to the application of isotopic replacement methods in complementary high-angle neutron fibre diffraction studies of DNA hydration[4,5,6,7]. Further insight into the factors which stabilise particular DNA conformations are being obtained from diffraction studies of chemically modified DNA in which particular base atoms are changed so that the relative importance of various DNA/ion/water interactions can be investigated in a very specific way. The first of the two examples described below concerns one such modification which is being used to probe the significance of major groove interactions in stabilising particular DNA conformations.

These applications of fibre diffraction place heavy demands on data processing both from the point of view of the development of good algorithms and also in terms of the sheer volume of data that often requires processing. They have therefore benefitted enormously from the data measurement procedures currently available under CCP13 in which there has been a significant shift in emphasis away from interactive data processing methods. The development of these routines within a suite that is of wide application in fibre diffraction problems and which readily interfaces with other packages such as the CCP4 suite for protein crystallography and the BSL and OTOKO suites for non-crystalline diffraction is particularly important.

Methods All samples used for the work described here were made from DNA that was synthesised enzymatically for these studies. DNA synthesis was performed by incubation of the appropriate nucleoside triphosphates with DNA polymerase I in the presence of a DNA primer and magnesium chloride. Reaction mixtures were monitored by agarose gel electrophoresis and efficiencies measured using size exclusion chromatography. The final DNA product was purified and fibres drawn from concentrated gels using standard techniques.

Fibre diffraction data were recorded using the beamline 7.2 at the Daresbury SRS operating at a wavelength of 1.488A obtained using the (111) plane of a germanium monochromator. This instrument has recently been refurbished and is now fully optimised for biological studies using high-angle fibre diffraction and protein crystallography. The fibre diffraction arrangement operates using a purpose-designed camera which allows the humidity of the sample environment to be closely controlled and monitored in a helium environment that eliminates scatter due to air. The system available on beamline 7.2 allows for data collection using photographic film, an online "Mar Research" image plate detector, or an offline "Molecular Dynamics" image plate system. A mount also exists which will accommodate a Photonic Science CCD detector on the optical bench.

Data were processed using firstly the routine CONV to convert the data type to standard CCP13/BSL format. In the case of image plate data from the Molecular Dynamics system the routine SPD_COR[8] was used prior to this to correct for spatial distortion effects. The interactive programme FIX was used to determine standard pattern parameters required for further processing. The data were then mapped into reciprocal space using FTOREC, and finally intensities measured using LSQINT. These procedures allowed the production of datasets that could then be readily fed into the CCP4 suite where a large number of programmes for the processing of single crystal data are available.

The A form of Poly[d(c_7A-T)].poly[d(c_7A-T)] This DNA polymer has been studied over a wide range of salt strengths and exhibits conformational polymorphism that is completely different from that observed for unmodified poly[d(A-T)].poly[d(A-T)]. It does not, in any circumstances yet identified, adopt the D conformation. In conditions which would normally favour the D form for the unmodified polymer it instead adopts an unusual variant of A-DNA and at very low humidities a novel conformation that has not been reported previously. Figure 1 shows a diffraction pattern recorded from the A form of this polymer. The molecule packs into a monoclinic lattice in space group C2 having parameters a=22.82A, b=40.67A, c=27.74A, β=96.7°. The pattern exhibits clear differences in intensity distribution from that observed for classical A-DNA fibre diffraction patterns in the region of the 6th, 7th and 8th layer lines.

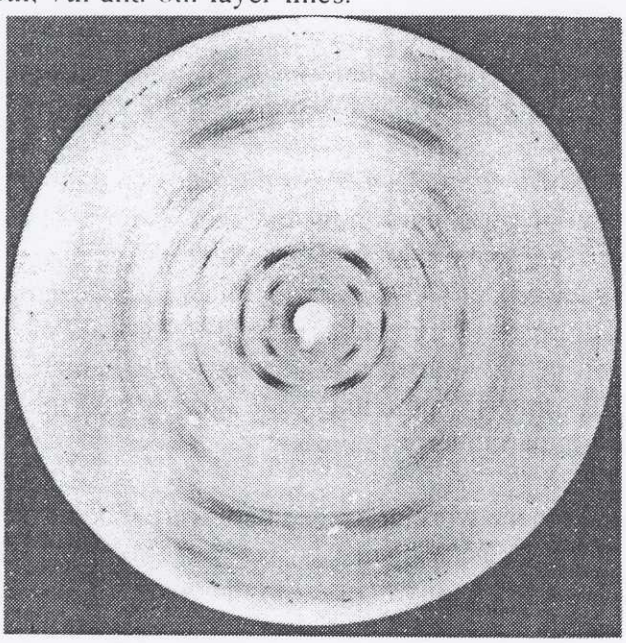


Figure 1: High-angle x-ray fibre diffraction pattern recorded from the A form of poly[d(c_7A-T)].poly[d(c_7A-T)]

Figure 2(a) shows the reciprocal space image produced from the data shown in Figure 1 using the routine FTOREC. The processing of FTOREC images using LSQINT requires reasonable starting values for the lattice parameters; these were obtained using FIX. The NOFIT option available within LSQINT was found to be particularly useful in estimating initial disorder parameters. The dataset shown in Figure 2(a) was measured by repeated applications of LSQINT using the maximum entropy (MAXENT) intensity fitting option available within the programme. Background was fitted to the pattern using the BACK WINDOW option. As with most A-DNA fibre diffraction patterns, data measurement was complicated by the effect of so-called "double orientation" which results in a slight displacement of some Bragg reflections either above or below the layer lines. This effect was well handled in LSQINT using the MISSETTING option which allowed a missetting angle to be refined alongside other parameters. The final fit to the observed data is seen in Figure 2(b) which shows a simulation of the pattern based on the fitted intensities. The agreement residual comparing the observed and the simulated data was 21%. The intensities measured from this pattern are currently being used to undertake a linked atom least squares (LALS[9]) analysis of this A-DNA helix.

(a) (b)

Figure 2: (a) the FTOREC image obtained from the raw data shown in Figure 1; (b) an image simulated from the data measured by LSQINT.

The Thallium salt of the D-DNA Double Helix High-angle x-ray fibre diffraction patterns have recently been recorded from a thallium salt of the D-DNA double helix. The scattering power of the Tl^+ (Z=81) cation in this derivative is markedly larger than was obtained in previous studies where difference Fourier methods based on data recorded from the Li^+, Na^+, K^+, and Rb^+ salts of D-DNA were used to image cation locations around previously refined models for the DNA[10,11]. A thallium D-DNA diffraction pattern is shown in Figure 3. The D-DNA molecules form a centred orthorhombic array in space group $C222_1$ with lattice parameters a=23.45A, b=25.32A, c=24.30A. The distribution of intensity throughout the pattern is dramatically different from that recorded for other derivatives of D-DNA.

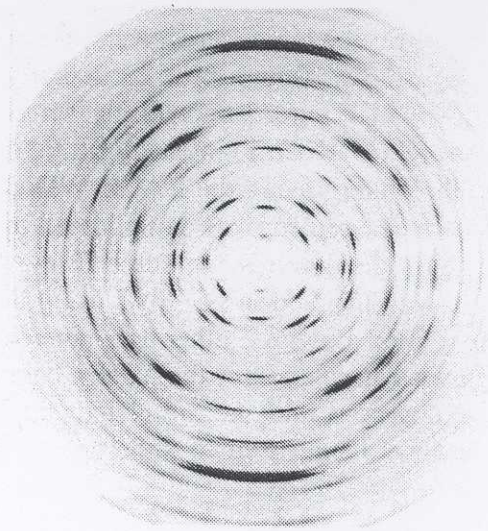
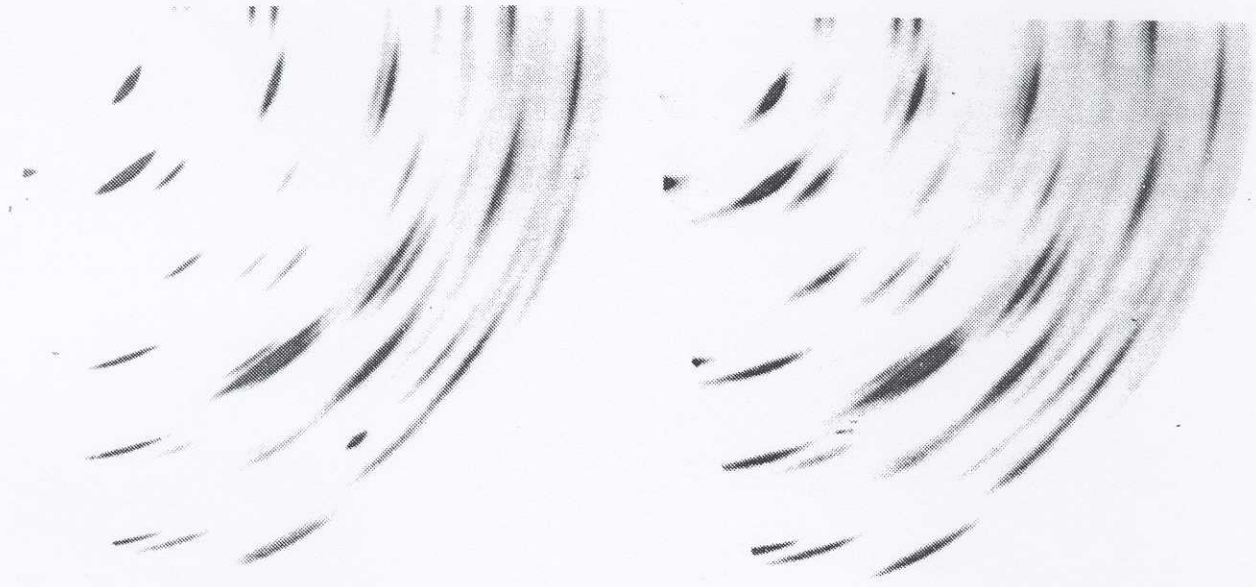


Figure 3: High-angle x-ray fibre diffraction pattern recorded from the thallium salt of D-DNA.



(a) (b)

Figure 4: (a) the FTOREC image obtained from the raw data shown in Figure 3; (b) an image simulated from the data measured by LSQINT.

Figure 4(a) shows the reciprocal space image of this pattern as produced by FTOREC. The processing of this dataset with LSQINT followed essentially the same path as described for the previous example. However in this case the least-squares option of intensity fitting was used rather than the maximum entropy option. It was also found that in this case better results were obtained using the GLOBAL background fitting option. The final agreement residual was 24%. A Patterson analysis of this data is currently in progress.

Conclusion The examples described above demonstrate the efficacy of the programmes available within CCP13. It is clear that the resulting improvements in the accuracy with which data can be measured using these routines will have a major impact on the quality of fibre diffraction analyses

both for definitive structural work on "static" DNA conformations and also for the mapping of the stereochemical pathways followed during structural transitions using time-resolved high-angle x-ray fibre diffraction. The development and rationalisation of fibre diffraction software within CCP13 is being paralled by a number of experimental developments which are occurring for x-ray and also for neutron high-angle fibre diffraction work at central facilities such as the Daresbury Laboratory SRS, the Institut Laue-Langevin reactor neutron source, and the ISIS spallation neutron source. The use of these facilities for fibre diffraction has a wide range of implications for data processing methods and it can be expected that CCP13 will provide an important framework within which such methods can be developed.

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References

1. Mahendrasingam, A., Forsyth, V.T., Hussain, R., Greenall, R.J., Pigram, W.J., Fuller, W., *Science* 233, 133 (1986).
2. Forsyth, V.T., Greenall, R.J, Hussain, R., Mahendrasingam, A., Nave, C., Pigram, W.J., and Fuller, W., *Biochem. Soc. Trans.* 14, 533 (1986).
3. Mahendrasingam, A., Denny, R.C., Forsyth, V.T., Greenall, R.J., Pigram, W.J., Papiz, M.Z., and Fuller, W., *Inst. Phys. Conf. Ser.* 101, 225 (1989).
4. Forsyth, V.T., Mahendrasingam, A., Langan, P., Pigram, W.J., Stevens, E.D., Al-Hayalee, Y.A., Bellamy, K.A., Greenall, R.J., Mason, S.A., and Fuller, W., *Int. J. Biol. Macr.* 11, 236, (1989).
5. Fuller, W., Forsyth, V.T., Mahendrasingam, A., Pigram, W.J., Greenall, R.J., Langan, P., Bellamy, K.A., Al-Hayalee, Y.A., and Mason, S.A., *Physica B* **156/157**, 468 (1989).
6. Langan, P., Forsyth, V.T., Mahendrasingam, A., Pigram, W.J., Mason, S.A., and Fuller, W., *J. Biomol. Str. Dyn.* 10(3), 489 (1992).
7. Langan, P., Forsyth, V.T., Mahendrasingam, A., Alexeev, D., Fuller, W., and Mason, S.A. *Physica B* 180/181, 759 (1992).
8. Hammersley, A.P., Svensson, S.O., Thompson, A., *Nucl. Inst. Meth.* A346, 312 (1994).
9. Smith, P.J.C., and Arnott, S., *Acta Cryst.* A34, 3 (1978).
10. Forsyth, V.T., Mahendrasingam, A., Langan, P., Pigram, W.J., Stevens, E.D., Al-Hayalee, Y.A., Bellamy, K.A., Greenall, R.J., Greenall, R.J., Mason, S.A., and Fuller, W., *Inst. Phys. Conf. Ser.* 101, 237 (1989).
11. Forsyth, V.T., Langan, P., Mahendrasingam, A., Mason, S.A., and Fuller, W., *Proc. Ital. Phys. Soc.* 43, 231 (1993).